

CLAIMS

1. A method of obtaining a specific binding pair (sbp) member that binds a complementary sbp member of interest, the  
5 method comprising:

(a) providing mRNA molecules, each mRNA molecule comprising a nucleotide sequence encoding a specific binding pair member and lacking an in-frame stop codon;

(b) incubating the mRNA molecules under conditions for  
10 ribosome translation of the mRNA molecules to produce encoded specific binding pair member, whereby complexes each comprising ribosome, mRNA and encoded specific binding pair member displayed on the ribosome are formed;

(c) bringing the complexes into contact with the  
15 complementary sbp member of interest, and selecting one or more complexes displaying specific binding pair member able to bind the complementary sbp member of interest under the conditions of the selection;

wherein the mRNA molecules are incubated with prokaryotic  
20 ribosomes in a prokaryotic ribosome display system or are incubated with eukaryotic ribosomes in a eukaryotic ribosome display system;

the method being characterised in that the mRNA molecules further comprise a sequence for encapsidation of the mRNA  
25 molecules in a viral coat, and the method comprises providing viral coat protein that recognises the sequence for encapsidation, thereby encapsidating complexes of mRNA, ribosome and displayed specific binding member in the viral coat protein.

30

2. A method according to claim 1 wherein the mRNA molecules incorporate a Midvariant (MDV) RNA template enabling replication by Q $\beta$  replicase.

3. A method according to claim 1 wherein a gly-ser tether is fused C-terminally to specific binding pair member.

5 4. A method according to claim 3 wherein the gly-ser tether comprises 24 glycine-serine units.

5. A method according to claim 1 wherein oxidised and reduced glutathione is added at a ratio of between 1:1 and  
10 10:1 after 30 minutes of ribosome translation.

6. A method according to claim 1 wherein protein disulphide isomerase (PDI) is employed in the incubation conditions, along with oxidised and reduced glutathione at a ratio of 1:1  
15 and 10:1.

7. A method according to claim 1 wherein the translation system is eukaryotic and protein disulphide isomerase (PDI) is employed in the incubation conditions.  
20

8. A method according to claim 1 comprising selecting for complexes comprising a specific binding member able to bind complementary specific binding member of interest, while blocking unspecific selection using heparin.  
25

9. A method according to claim 1 wherein mRNA molecules for incubation in the translation system are provided by means of RT-PCR reactions in which at least one RT-PCR primer is a mutagenic primer encoding a diversity of different sequences  
30 for inclusion in a defined region of the nucleotide sequence encoding a specific binding pair member.

10. A method according to claim 1 wherein tobacco mosaic

11. A method according to claim 1 further comprising

12. A method according to claim 11 wherein mRNA retrieved from a selected complex displaying a specific binding pair member (a "selected specific binding pair member") is

13. A method according to claim 12 wherein the DNA is provided in an expression system for production of a product, which product is the selected specific binding pair member or a polypeptide chain of the selected specific binding pair member.

15. A method according to ~~claim 14~~ further comprising formulating the product into a composition comprising at least one additional component.

16. A method according to claim 15 wherein DNA encoding the selected specific binding pair member or a polypeptide chain of the selected specific binding pair member is provided within a nucleotide sequence to provide a nucleotide sequence encoding a fusion protein comprising the selected specific binding pair member, or a polypeptide chain of the selected specific binding pair member, fused to additional amino acids.

17. A method according to claim 16 wherein the selected specific binding pair member comprises an antibody VH and/or antibody VL domain and the additional amino acids comprise an antibody constant domain.

18. A method according to claim 16 wherein DNA comprising said nucleotide sequence encoding said fusion protein is provided in an expression system for production of a product, which product is the fusion protein.

19. A method according to claim 18 further comprising isolating or purifying the product.

21. A method according to ~~claim 12~~ wherein DNA encoding the selected specific binding pair member or a polypeptide chain of the selected specific binding pair member is mutated to encode a polypeptide that comprises an amino acid sequence that differs from the selected specific binding pair member or polypeptide chain of the selected specific binding pair member.

22. A method according to ~~claim~~ 21 wherein mutated DNA encoding said polypeptide is provided in an expression system for production of a product, which product is said polypeptide.

23. A method according to claim 22 further comprising isolating or purifying the product.

24. A method according to claim 23 further comprising  
formulating the product into a composition comprising at least  
one additional component.

25. A nucleic acid construct which is DNA or RNA comprising the following elements: an RNA polymerase binding site, a Kozak consensus sequence, a ribosome binding site, an initiation codon, a coding sequence encoding a fusion protein comprising a polypeptide and a tether, the coding sequence lacking a termination codon, and a sequence for encapsidation of mRNA in a viral coat.

26. A library or population of RNA molecules in accordance with claim 25, each RNA molecule in the library or population containing a sequence encoding a specific binding pair member, wherein the library or population collectively encodes a population or repertoire of specific binding pair members of diverse sequence.

20 27. A library or population of RNA molecules according to  
claim 26 which is packaged within viral coat protein.

28. A population of viral particles collectively containing a population or library of RNA molecules according to claim 27.

25

29. An expression system comprising a nucleic acid construct according to ~~claim 25~~ under culture conditions for production of fusion protein comprising polypeptide and tether.

30 30. An expression system comprising a library or population  
according to claim 26 under culture conditions for production  
of fusion protein comprising polypeptide and tether.